

[illegible]

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A)

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(d) a fourth sequence, at the 5' end of the first primer, which is complementary to the second sequence,

under hybridization conditions sufficient for annealing the first sequence of the first primer to the sequence at the 3' end of the single-stranded cDNA, and

(iii) a polymerase;

B) incubating the mixture from step (A) under conditions for DNA synthesis; and

C) performing a polymerase chain reaction by adding

(i) an aliquot of the mixture from (B),

(ii) a second primer which specifically binds to single-stranded cDNA,

(iii) a third primer which comprises

(a) a fifth sequence identical to the sequence of the first primer, and

(b) a sixth sequence identical to the sequence of the second sequence of the primer, and

(iv) a polymerase

under conditions suitable for a polymerase chain reaction as to produce a double-stranded cDNA reaction thereby isolating the cDNA having the sequence complete open reading frame.

2. The method of claim 1, wherein the single-stranded

B) incubating the mixture from step (A) under conditions for DNA synthesis; and

5 C) performing a polymerase chain reaction by admixing

(i) an aliquot of the mixture from (B),

10 (ii) a second primer which specifically binds to the single-stranded cDNA,

(iii) a third primer which comprises

15 (a) a fifth sequence identical to the 5' sequence of the first primer, and

(b) a sixth sequence identical to the 3' sequence of the second sequence of the

20 primer, and

(iv) a polymerase

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25

2. The method of claim 1, wherein the single-stranded cDNA is

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thereby isolating the cDNA having the sequence
complete open reading frame.

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under conditions suitable for a polymerase chain reaction
25 as to produce a double-stranded cDNA reaction
thereby isolating the cDNA having the sequence
complete open reading frame.

2. The method of claim 1, wherein the single-stranded

(b) a sixth sequence identical to a
of the second sequence of the
primer, and

(iv) a polymerase

under conditions suitable for a polymerase chain reaction
as to produce a double-stranded cDNA reaction
thereby isolating the cDNA having the sequence
complete open reading frame.

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under conditions suitable for a polymerase chain reaction as to produce a double-stranded cDNA reaction thereby isolating the cDNA having the sequence complete open reading frame.

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2. The method of claim 1, wherein the single-stranded

2. The method of claim 1, wherein the single-strand

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is a 5' portion of a cDNA reverse transcribed from an mRNA.

5 3. The method of claim 1, wherein the first primer has the sequence

3'-

NNNNNNNNNNNNNCAGAGCTCAAATTTGTGATCAGCTGGTCTTTCACAAATTTGAGCTC
TG-5' (D-SLAP).

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10 4. The method of claim 1, wherein the first primer has the sequence

3' NNNNNNNNNNNGGAGAGCTCACAGCTGAAGCAGCTGACTAGCACCTAGTGTAGAAT
ACATCTTGAGCTAT-5' (D-CLAP1).

15 5. The method of claim 1, wherein the first primer has the sequence

3' NNNNNNNNNNNNNNAGAGCTCACAGCTGAAGCAGCTGACTAGCACCTAGTGT
AGAATACATCTTGAGCTAT (D-CLAP2)

20 6. The method of claim 1, wherein the first primer comprises an inosine nucleotide.

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7. The method of claim 1, wherein the loop structure is a simple loop structure, or a cloverleaf loop structure.

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8. A method for generating a cDNA library which comprises:

A) admixing

B

(i) an aliquot of the mixture from (B),

(iii) a ~~third~~ primer which comprises

(b) a sixth sequence identical to a portion of the second sequence of the first primer, and

9. The method of claim 8, wherein the single-stranded DNA
25 is a cDNA reverse transcribed from an mRNA.

10. The method of claim 8, wherein the first primer has the sequence 3'-
NNNNNNNNNNNNCAGAGCTCAAATTTGTGATCAGCTGGTCTTTCACAAATTG

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AGCTCTG-5' (D-SLAP).

11. The method of claim 8, wherein the first primer has the sequence

5 3'NNNNNNNNNNNGGGAGAGCTCAGAGCTGAAGCAGCTGACTAGCACCTAGTGTAGAAT
ACATCTTGAGCTAT-5' (D-CLAP1).

12. The method of claim 8, wherein the first primer has the sequence

10 3'NNNNNNNNNNNNNAGAGCTCAGAGCTGAAGCAGCTGACTAGCACCTAGTGT
AGAATACATCTTGAGCTAT (D-CLAP2).

13. The method of claim 8, wherein the first primer comprises an inosine nucleotide.

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14. The method of claim 8, wherein the loop structure is a simple loop structure, or a cloverleaf loop structure.

15. A kit for the generation of a complete open reading
20 frame double-stranded cDNA of interest which comprises:

(i) a first primer capable of forming a stem-loop structure, comprising

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(a) at the 3' end of the primer, a first random sequence linked to

(b) a second sequence, linked to

(c) a third sequence which forms a loop

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structure, linked to

- (d) a fourth sequence, at the 5' end of the first primer, which is complementary to the second sequence, and

(ii) a second primer which comprises

- (a) a fifth sequence identical to the third sequence of the first primer, linked to
(b) a sixth sequence identical to a portion of the second sequence of the first primer.

16. A method for isolating a double-stranded cDNA having a nucleotide sequence of a complete open reading frame which comprises:

(a) admixing

(i) a biological sample containing mRNA,

(ii) a primer which forms a stem-loop structure, comprising:

(a) a poly-T sequence at the 3' end of the primer linked to

(b) a first random sequence linked to

(c) a second sequence which forms a loop

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structure linked to

5 (d) a third sequence at the 5' end of the primer
which is complementary to the first
sequence, and

(iii) a reverse transcriptase,

10 under hybridization conditions sufficient for annealing
the primer to the mRNA poly-A sequence;

(b) incubating the mixture from step (a) under suitable
conditions for reverse transcription;

15 (c) performing a polymerase chain reaction with an aliquot
of the mixture from step (b) using one gene-specific
primer which is pre-defined and one primer which has a
sequence identical to at least a portion of the primer
sequence of element (ii), thereby isolating the cDNA
20 having the sequence of the complete open reading frame.

25 17. The method of claim 16, wherein the primer has the
s e q u e n c e 3 ' -
TTTTTTTTTTTCAGAGCTCAAATTTGTGATCAGCTGGTCTTTCACAAATTTG
AGCTCTG-5' (T-SLAP).

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